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# ALTERNATIVE AND GENERALIZED APPROACH TO *IN VITRO–IN VIVO* CORRELATION

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**Abstract:** Evaluation of *in vitro–in vivo* correlation (IVIVC) plays important role in securing therapeutic effect if a dosage form undergoes technological modifications. Similarity (closeness) of dissolution profiles of the original and modified dosage forms has been traditionally considered to be sufficient for similar *in vivo* responses. This may be true if the IVIVC model (dependence between the dissolution and corresponding absorption profiles) is given by a linear straight line with the unit slope. The paper presents an alternative and generalized approach to IVIVC evaluation. Influences of pre-systemic processes (disintegration, dissolution, absorption) on the system response (concentration time profile C(t), bioavailability BD and other) are analyzed and evaluated. Both the magnitude and sign of IVIVC are then derived from the magnitudes and signs of these influences. The underlining idea is that pre-systemic processes do not correlate with the system response, (e.g., plasmatic concentration) if small modifications of the former do not induce significant changes of the later. If this is so, the therapeutic effects of the modified and original dosage forms may be considered equal or at least similar. In this way the problem of IVIVC is not only exactly mathematically founded but modifications of pre-systemic processes are directly projected to the system output – the time profile of plasmatic concentration. Moreover, the approach is applicable to virtually any dosage form. Its feasibility was validated *in vivo*.

**Keywords:** *in vitro–in vivo* correlation, influence analysis, generalized approach

Drug development and manufacturing may require altering the dosage form composition. Traditionally, if some modifications of dosage form were done, bioavailability studies would also have to be performed in many instances to ensure that the "new" drug form will exhibit similar *in-vivo* behavior as the original one. For that reason, a main objective of IVIVC evaluation is to decide whether the dissolution test may serve as a surrogate for bioavailability studies or not. A basic precondition is that dissolution conditions should be fixed for all formulations tested. Let us note that the correlation is a mathematical relationship and as such it will be processed in this study. In general, the correlation does not infer causal relationships. On the other hand, this does not mean that correlations cannot indicate potential existence of causal relations. That is just the case of IVIVC which indicates both mathematical and causal relationships. For instance, 90% correlation between the dissolution and absorption profiles means that these two profiles are virtually identical (mathematical relationship). But changes of the drug dissolution/release also induce changes of drug absorption (causal relationship) and therefore, under certain conditions, the dissolution test may be used as a surrogate of *in vivo* studies.

The *in vitro–in vivo* correlation is not quite exactly and unambiguously defined in pharmaceutical literature. (1–4). Losely speaking, it means a relation between the drug dissolution and the corresponding *in vivo* responses like the absorption, concentration-time profile – C(t), area under the curve – AUC, bioavailability, mean residence time – MRT etc. Traditional approaches to IVIVC evaluation frequently employ numerical deconvolutions (5) of the concentration-time profile yielding the absorption-time profile. The absorption-time profile is subsequently compared with the *in vitro* dissolution-time profile and a degree of similarity between these two profiles is evaluated. The obtained degree of similarity creates a ground for assessment of the correlation between *in vivo* and *in vitro* processes.

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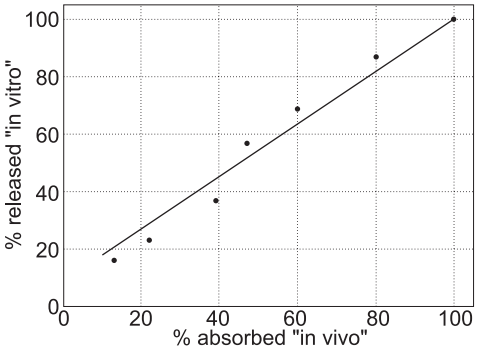

Figure 1. Linear IVIVC model

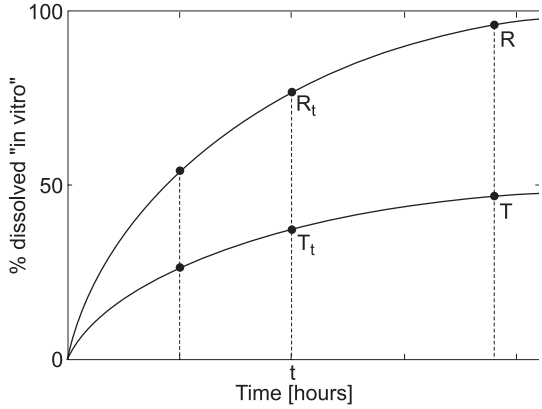

Figure 2. Comparison of two dissolution profiles.

Due to existence of complex dynamic relations (expressed by differential equations) between the drug dissolution and the corresponding plasmatic concentration, the scientists and pharmaceutical technologists have been satisfied with an acceptable degree of similarity between the dissolution profile of an original dosage form and that belonging to the modified dosage form. Their similarity has been evaluated by the difference factor $f_1$ or the similarity factor $f_2$. (6) Though such simplification of the reality saves time, money and many experimental animals that would otherwise fall victims, this procedure is possible on condition of rapid absorption when the absorption profile copies the dissolution one. For that reason, the so-called IVIVC model (dependence of a fraction of the drug absorbed on fraction of the drug dissolved in the same time) is sometime built (Fig. 1). The graphical representation of this dependence is a straight line with unit slope indicating that the dissolution and absorption rates are equal and eventual changes appearing in the rate of dissolution are instantly copied into the rate of absorption. In other words, no reservoir of the dissolved but not yet absorbed drug exists. Thus, it is just the rapid absorption what substantiates the similarity evaluation based on the difference factor $f_1$ or similarity factor $f_2$ (Fig. 2):

$$f_1 = \{(\sum_{t=1}^{n} |R_t - T_t|)/\sum_{t=1}^{n} R_t\} \times 100 \quad (1)$$

$$f_2 = 50\log\{[1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \times 100\} \quad (2)$$

The symbols $R_t$ and $T_t$ are samples of the reference and tested dissolution profiles, respectively, taken at time "t", "n" is a number of samples and log stands for decimal logarithm. The $f_1$ and $f_2$ factors are positive dimensionless quantities no greater than 100, bearing information about the degree of similarity between the reference and tested dissolution profiles. For example, if $f_2 = 100$ the dissolution profiles are equal, and $f_2 = 50$ means that there exist 10% difference between all samples.

Moreover, besides potential existence of the reservoir which inevitably inserts an additional kinetics, some sub-processes (drug inactivation, saturation mechanisms etc.) may run between the drug dissolution and its appearance in the blood circulation. Due to them, the global drug kinetics may become nonlinear and rather complicated. In view of these factors, the natural question arises: how large the mismatch between dissolution data of the original and modified dosage form may be to preserve the predictive power with regard to prediction of *in vivo* variations?

No wonder that the Biopharmaceutic Classification System (BCS) classifies the drug substances into four classes:
Class 1: high solubility – high permeability
Class 2: low solubility – high permeability
Class 3: high solubility – low permeability
Class 4: low solubility – low permeability,
and the Guidance For Industry (7) justifies biowaivers for Class 1 and partially for Class 3 of instantaneously released (IR) solid oral drug forms. This is because the use of (approved!) excipients in formulation of the substances of Class 1 does not affect absorption.

**Alternative approach**

Contrary to the evaluation of the similarity between dissolution profiles, the approach adopted in this study employs the concept of sensitivity, namely the sensitivity of a given *in vivo* metrics to modifications of pre-systemic parameters. The concept of sensitivity and the derived results are related to the whole process starting by the disintegration and ending by the drug elimination from the body. The leading idea is based on a simple true: The profile of

plasmatic concentration will remain unchanged by technological modifications of a dosage form if it is insensitive to those modifications. Therefore, based on the concept of sensitivity, it always makes sense to analyze conditions under which the *in vivo* response remains untouched by technological modifications of the dosage form. Specifically, if the *in vivo* response is insensitive to modifications of dissolution profile then the modified dosage form is very probably bioequivalent to the original (non-modified) one and the dissolution test may be considered as a waiver of *in vivo* studies. That is just a core of problems this study deals with.

To manage the problem, it is applied a system approach. The relations between the drug disintegration, dissolution and absorption on one side and the biological response on the other side are modelled by equations and sensitivities of a biologic response (plasmatic concentration) to variations of pre-systemic processes are evaluated. The system approach is fully justified because besides the rate and extent of the dissolution, there is a number of other parameters determining the *in vivo* response. To mention only a few, it can be a degree of the drug granulation (particle size), the transport across biological membranes, the first pass effect and others. As a consequence, the direct relations between *in vitro* dissolution and *in vivo* performance are far from straightforward.

To our knowledge, means establishing direct relationships between modifications of the *in vitro* dissolution and the induced modifications of *in vivo* performance have not been analyzed in the realm of IVIVC research. In this view, the approach presented here may serve as a supporting utility for decision making in the field of IVIVCs. The approach is based on a predictive model having capacity to predict both directions and magnitudes of deviations of the drug amount (or concentration) in the blood incurred by deviations of dissolution parameters from nominal values. The approach derives from the theory of dynamic sensitivity (8).

**Concept of sensitivity functions**

Let us suppose the n-compartment pharmacokinetic model (1) described by the set of differential equations of the first order:

$$\frac{dM_i(t)}{dt} = F_i(M_1, M_2,...M_n,q_1, q_2,...q_m) \qquad (3)$$

where $F_i$ means a (nonlinear) function related to the i-th compartment, $M_i(t)$ means a drug amount in the i-th compartment at time "t", n is a number of compartments and m is a number of parameters $q_i$.

The sensitivity function S (in short "sensitivity") of the amount $M_i$ to deviations of the parameter $q_j$ is defined as the partial derivation of $M_i$ with respect to the parameter $q_j$:

$$S_{M_i, q_j} = \frac{\partial M_i}{\partial q_j} \qquad (4)$$

For instance, the sensitivity of $M_2$ to deviations of the absorption rate constant $K_a$ is written as a partial derivation of $M_2$ w.r.t. $K_a$, i.e.:

$$S_{M_2, K_a} = \frac{\partial M_2}{\partial K_a} \qquad (5)$$

As follows from eq. (4), the unit of the sensitivity function is given by the unit of drug amount divided by the unit of the corresponding parameters. All (n × m) sensitivities may be obtained by solving the following set of (n × m) differential equations, known as a *sensitivity model*, for unknown sensitivities S:

$$\frac{dS_{M_i, q_j}}{dt} = \sum_{i=1}^{n} \frac{\partial F_K}{\partial M_i} S_{M_i, q_j} + \frac{\partial F_K}{\partial q_j}$$
$$k = 1, 2,\ldots n; \; j = 1, 2,\ldots \qquad (6)$$

The initial conditions of the equations (6) are set to zeroes. Having the sensitivities computed, the computer can calculate deviations $\Delta M_i(t)$ caused by the deviations $\Delta q_j$ of the parameter $q_j$.

$$\Delta M_i(t) = S_{M_i, q_j}(t)\Delta q_j \; i = 1, 2,\ldots n, \; j = 1, 2,\ldots m, \qquad (7)$$

and the new (deviated) value of $M_i$ at time "t" is given by the expression:

$$M_i(t)_{new} = M_i(t) + \Delta M_i(t) \qquad (8)$$

**Illustrative example**

To illustrate the methodology, in what follows the sensitivity analysis of the two-compartment model (1) of e.v. application of a liquid drug is presented. The liquid drug has been chosen intentionally to avoid the need of concatenating partial sensitivities (the problem that is supposed to be analyzed in the sequel paper). The site of application is supposed to exhibit the first order absorption. After application of the dose $M_0$ at time t = 0 the drug amounts $M_1$ and $M_2$ in the compartments 1 and 2 will evolve in time in accordance with the differential equations (1):

$$\frac{dM_1}{dt} = -(K_{12} + K_e)M_1 + K_{12}M_2 + K_aM_0\exp(-K_at)$$
$$\frac{dM_2}{dt} = K_{12}M_1 - K_{21}M_2 \qquad (9)$$

where $K_a$ and $K_e$ are absorption and elimination rate constants, respectively, $K_{i,j}$ (i = 1, 2, j = 1, 2) are rate constants of the drug flow from i-th to j-th compartment and "t" is time. The last term in the first equation reflects exponential decrease of the drug

amount on the site of application. Sensitivities $S_{Mi,Ka}$ (i = 1, 2) of the amounts $M_1$ and $M_2$ to change of absorption rate constant $K_a$ will be obtained as solutions of the following sensitivity model:

$$\frac{dS_{M_1,K_a}}{dt} = -(K_{12} + K_e) + K_{12}S_{M_2,K_a} + M_0 - t \cdot K_e \cdot \exp(-K_a \cdot t) \quad (10)$$

$$\frac{dS_{M_2,K_a}}{dt} = K_{12}S_{M_1,K_a} - K_{21}S_{M_2,K_a}$$

Equations (10) were derived from (9) by derivation w.r.t. the parameter $K_a$ and applying the denotation (4). The differential equations (9) and (10) jointly represent the so-called *augmented model*.

## RESULTS AND DISCUSSION

This paragraph illustrates a way of employing the augmented model for prediction of both the direction and extent (magnitude) to which the time profile of the drug amount $M_1$ will deviate from its nominal time profile as a result of the increased absorption rate constant $K_a$. To this end, the solution of natrium p-aminosalicylicum was first applied to rats and after a washout period, the experiment was repeated with added tenside Tween 80, which is known to increase the absorption rate constant $K_a$. *In vivo* samples of plasmatic concentration were used for identification of model parameters by using theory of adaptive models (9) and the following values were obtained: $K_a = 4\ h^{-1}$, $K_e = 0.98\ h^{-1}$, $K_{12} = 2.06\ h^{-1}$, $K_{21} = 0.61\ h^{-1}$ (10). The augmented model was then solved for two unknowns, namely the plasmatic concentration $C_1$ and the sensitivity function $S_{Ka}$. The time profiles obtained are shown in Figure 3. The curves A and B represent the time profile of the drug concentration $C_1$ as obtained *in vivo* without Tween 80 (dashed curve B) and with Tween 80 (solid curve A). For simulation purposes, the distribution volume V was set to unit value to avoid the need to plot $C_1$ and $M_1$ separately. The time course of the sensitivity function $S_{ka}$ (sensitivity of $C_1$ to deviations of $K_a$) is shown in the upper part of Figure 3.

It can be seen that the sensitivity $S_{ka}$ is positive over the time interval approx. 0 – 0.8 h, then it falls below zero and remains negative. On the other hand, the time course of $S_{ka}$ predicts the system behavior in the following sense: the increase of $K_a$ (caused by Tween 80) increases $C_1$ (curve A ) at the beginning, but after 0.8 h the concentration $C_1$ falls under the dashed nominal curve B (without Tween). Clearly, the actual and predicted system behavior is the same. This can be observed from the curves A and B, which were obtained from the *in vivo* experiment. Thus, the behavior predicted by sensitivity function actually matches that of observed *in vivo*. Moreover, the $S_{ka}$ correctly predicts not only magnitudes but also directions of the changes, which the $C_1$ undergoes as a result of the deviated absorption rate constant $K_a$. In particular, the change of $C_1$ at time "t" caused by the deviation $\Delta K_a$ is equal to $\Delta C_1(t) = S_{ka}(t) \times \Delta K_a$, so the sign of $\Delta C_1(t)$ depends both on the signs of $S_{ka}(t)$ and $\Delta K_a$.

In general, the low magnitude of sensitivity function indicates that the plasmatic concentration is only minimally influenced by the deviation of a corresponding pre-systematic parameter, or in other words, it indicates that the plasmatic concentration is minimally sensitive to changes of that pre-systemic parameter. In this view, the sensitivity function, as defined above, may be considered as a good quantitative measure of IVIVC. In particular, the lower sensitivity of the drug concentration profile to modifications of dissolution profile the higher chance exists for dissolution test to be used as a waiver of *in vivo* studies. Said in other words, if modifications of the dissolution profile should not significantly influence the plasmatic concentration profile, its sensitivity w.r.t. deviations of dissolution profile should be low. The fact that the behavior predicted by the sensitivity function was found equivalent to that of observed *in vivo* is also an indirect proof of the correctness of compartment model, i.e., the model correctly reflects an actual biological state.

## CONCLUSIONS

An alternative approach to IVIVC evaluation was presented. In comparison with traditional dissolution tests it possesses the following advantages:

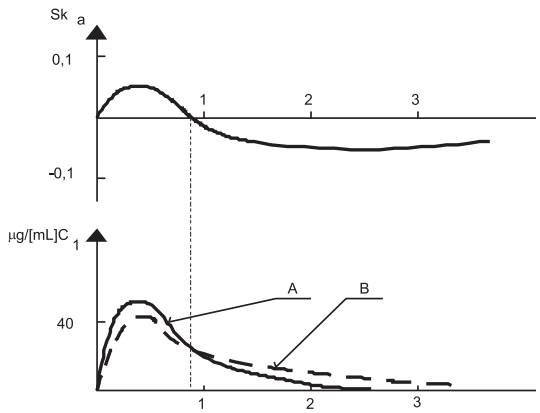


Figure 3. Time courses of $S_{ka}$ and $C_1$ after (curve A) and before (curve B) addition of Tween 80

The drug dissolution/release has been commonly considered as the rate-limiting process. Due to this, the time profiles of the dissolution and absorption rates are considered equal though this may not be true. Under certain circumstances, the dissolved drug may remain temporally trapped in the site of application what introduces an additional kinetics (and not only a delayed absorption). Such cases can be easily addressed by the presented method.

The method can be easily applied on any dosage form regardless of the dissolution mechanisms and/or dissolution profiles (zero-order, first-order, Weibull, Hixon-Crowell, Higuchi, logistic, etc.) (4) or in case of sophisticated (programmable, intelligent) drug delivery systems. Moreover, the approach is directly applicable on a dose-dependent (nonlinear) kinetics. The reader is advised to look at eq. (3) where no restrictions were laid on the function F, which can be nonlinear.

An objection may be raised stating that the approach presented requires a correct pharmacokinetic model. This is true but it does not hinder application of the method because today exist modern adaptive and learning means which make the problem of building a correct model marginal. Adaptive models (including neural nets) are able to identify a correct population pharmacokinetic model a set of *in vivo* samples.

Issues related to analysing influences of a complex chain of pre-distribution processes, i.e., disintegration, dissolution and absorption on a chosen *in vivo* performance measure and prediction of *in vitro–in vivo* correlation may be presented in the sequel of this paper.

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